Plant Physiol. (1990) 94, 1214–1218
0032-0889/90/94/1214/05/$01.00/0

Received for publication March 27, 1990
Accepted June 27, 1990

Superoxide Dismutase, Catalase, and α-Tocopherol Content of Stored Potato Tubers[1]

James P. Spychalla*[2] and Sharon L. Desborough

Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Activated oxygen or oxygen free radical mediated damage to plants has been established or implicated in many plant stress situations. The extent of activated oxygen damage to potato (*Solanum tuberosum* L.) tubers during low temperature storage and long-term storage is not known. Quantitation of oxygen free radical mediated damage in plant tissues is difficult. However, it is comparatively easy to quantitate endogenous antioxidants, which detoxify potentially damaging forms of activated oxygen. Three tuber antioxidants, superoxide dismutase, catalase, and α-tocopherol were assayed from four potato cultivars stored at 3°C and 9°C for 40 weeks. Tubers stored at 3°C demonstrated increased superoxide dismutase activities (up to 72%) compared to tubers stored at 9°C. Time dependent increases in the levels of superoxide dismutase, catalase, and α-tocopherol occurred during the course of the 40 week storage. The possible relationship between these increases in antioxidants and the rate of activated oxygen production in the tubers is discussed.

During normal aerobic metabolism, ground state dioxygen can be chemically activated by a series of univalent reductions, yielding superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH). Oxygen can also be physically activated with an input of energy, yielding singlet oxygen ($^1O_2$) (7). These are the biologically important forms of activated oxygen that aerobes generate *in vivo* through autooxidations, enzymatic reactions, and leakage from membrane electron transport chains (7, 11, 14). Activated oxygen is highly reactive and can cause deleterious oxidations of lipids (11, 13–15, 19, 22, 24, 30), proteins (1, 3, 11, 13), and nucleic acids (11, 13, 16). Excessive oxidative damage to such molecules can seriously perturb normal cell metabolism.

Plants possess several tissue antioxidants for protection against the potentially cytotoxic forms of activated oxygen. Among the potato (*Solanum tuberosum* L.) antioxidants are SOD[3] (O_2^-:O_2^- oxidoreductase, EC 1.15.1.1), CAT (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6), and α-tocopherol (vitamin E). SOD scavenges the superoxide radical, the one electron reduced form of oxygen: $2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$. CAT scavenges hydrogen peroxide, the two electron reduced form of oxygen: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. α-Tocopherol protects membrane polyunsaturated fatty acids by termination of chain propagating free radical peroxidation within the membrane bilayer (7, 13, 14, 18, 19, 24, 30).

Environmental stress such as drought, excessive light, temperature extremes, air pollution, wounding, or herbicides can disturb normal cellular metabolism. This can upset the balance of oxygen free radical production and quenching, and tests the antioxidant capacity of the aerobic cell (3, 4, 7, 13, 21, 24, 26, 30). High constitutive levels or high induced levels of antioxidants in a plant cell may provide resistance to a particular stress (6, 15, 30). Likewise in these situations, one or more antioxidants may be increased, imparting resistance to damage from activated oxygen.

The quality of tubers for fresh market, processing, or as vegetative seed out of storage depends greatly upon the cultivar and the storage conditions (25). Commercially stored potato tubers are commonly held between 2 to 10°C for up to 10 months. Thus two stresses that stored potato tubers must endure are low temperature and aging. Our objective was to evaluate the antioxidative capacity of tubers from different cultivars undergoing these stresses.

MATERIALS AND METHODS

Growth and Storage of Potatoes

Potatoes (*Solanum tuberosum* L.) were grown and stored as described previously (29).

Enzymes and Chemicals

Cyt *c*, xanthine oxidase, xanthine, α-tocopherol, and α-tocopherol acetate were obtained from Sigma Chemical Co. Petroleum ether was of commercial grade. All other chemicals were of reagent grade.

Enzyme Extraction

Tuber tissue (40 g) was frozen in liquid nitrogen and stored at −20°C until extraction. The frozen sample was homogenized in a 1 L blender (Waring, New Hartford, CT) at 50% full speed in 250 mL of 0.1 M Na_2HPO_4/NaH_2PO_4 (pH 7.0) at 4°C for 2 min. The homogenate was centrifuged at 4°C for 15 min at 4000*g*. The supernatant was used for the CAT

[1] From a dissertation submitted to the graduate school of the University of Minnesota, by J. P. S. in partial fulfillment of the requirements for the Ph.D. degree. The research was supported by the Minnesota Agricultural Experiment Station and by a grant from the Frito-Lay Co., Inc. This is Minnesota Agricultural Experiment Station Paper No. 17,962.

[2] Present address: Molecular Genetics Department, Cambridge Laboratory, Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UJ, United Kingdom.

[3] Abbreviations: SOD, superoxide dismutase; CAT, catalase; gfw, gram of fresh weight.

assay. For the SOD assay, the supernatant was filtered (No. 1 Whatman). The low mol wt components of the filtrate were removed and the buffer exchanged by applying 1 mL of the filtrate to a gel filtration column (10 mm · 100 mm, Sephadex G-25 coarse) equilibrated in 50 mM $Na_2CO_3/NaHCO_3$ (pH 10.2).

SOD Assay

SOD activity in extracts of potato tubers was based upon the indirect spectrophotometric method of Forman and Fridovich (9). The assay was performed in a 3.0 mL cuvette at 25°C with a recording spectrophotometer (model DU-50, Beckman Instruments Inc.). The reaction mixture contained 50 mM $Na_2CO_3/NaHCO_3$ (pH 10.2), 0.10 mM EDTA, 0.01 mM ferricytochrome *c*, and 0.05 mM xanthine. The assay was initiated by the addition of sufficient xanthine oxidase to produce a basal rate of ferricytochrome *c* reduction corresponding to an increase in A_{550} of 0.035 units/min (V_1). After verification of V_1, tuber extract was added and the resulting reaction velocity (V_2) calculated. One unit of superoxide dismutase was defined as the amount of enzyme which inhibited the rate of ferricytochrome *c* reduction by 50% ($V_1/V_2 = 2$) in a 3 mL assay volume. Units of activity were calculated as a function of the reaction velocities (units = $V_1/V_2 - 1.06$).

Catalase Assay

CAT activity in extracts of potato tubers was determined with a polarographic oxygen sensor (model K-ICT-C oxygraph, Gilson Medical Electronics, Middleton, WI) according to the method of del Rio *et al.* (5). One unit of CAT activity was defined as producing 1 μmol O_2/min.

Protein Assay

Total soluble protein content in the enzyme extracts of potato tubers was determined by Coomassie dye binding (27). Bovine serum albumin was the protein standard.

α-Tocopherol Assay

Tuber tissue (100 g) was frozen in liquid nitrogen and stored at −20°C until extraction. The frozen tissue was homogenized for 2 min in a 1 L blender with 250 mL of 80% ethanol in water (v/v) which contained 750 μg of α-tocopherol acetate as an internal standard. The homogenate was centrifuged at room temperature for 5 min at 2500*g*, filtered (No. 1 Whatman), mixed with 25 mL of petroleum ether, and again centrifuged. The upper phase was partitioned and the solvent removed under vacuum at 50°C. The residue was dissolved with 0.7 mL of methanol and filtered (0.45 μm). Chromatography was performed at room temperature through a 10 μm particle C-18 Bondex stationary phase which consisted of a 4.6 mm · 50 mm guard column coupled to a 3.9 mm · 300 mm analytical column (Phenomenex, Torrance, CA). The mobile phase was 95% methanol in water (v/v) delivered at 2 mL/min. Sample volume was 200 μL. UV absorption of the column eluent was monitored at 295 nm and recorded as peak areas.

Experimental Design and Statistical Analysis

The experimental unit, experimental design and statistical analyses were as described previously (29).

RESULTS

The responses of the SOD, CAT, and α-tocopherol assays to sample size were determined to be linear in the assay range (Fig. 1, A–C). SOD and CAT activities were eliminated when the tuber enzyme extracts were heated at 95°C for 3 min. The

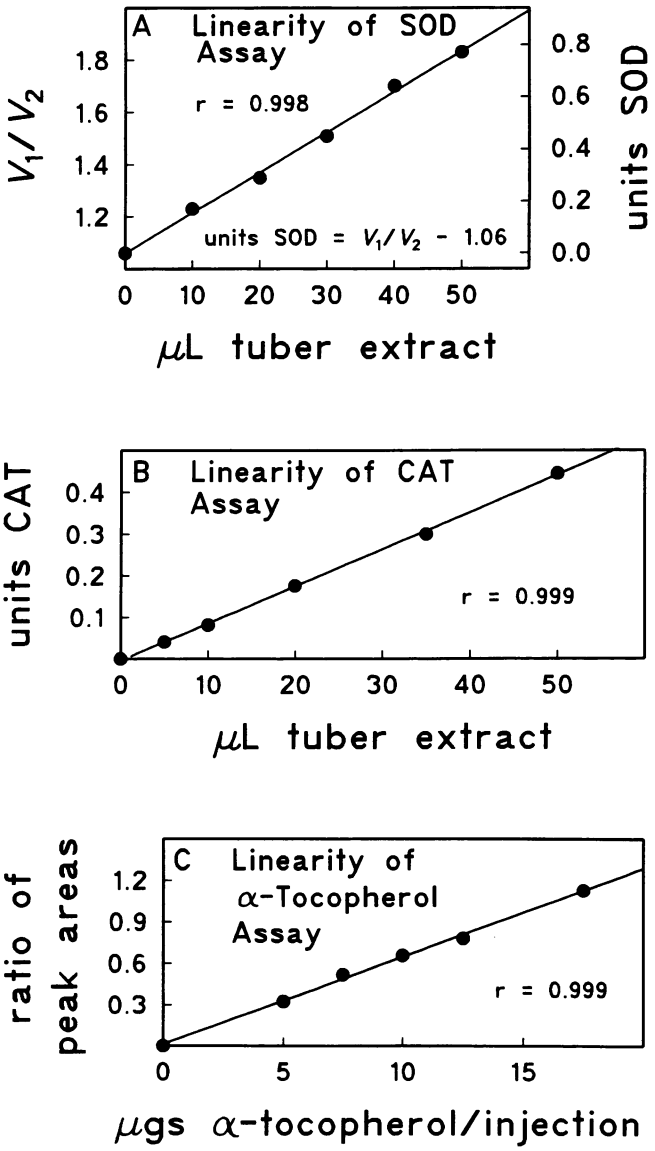

Figure 1. Linear response of (A) the SOD assay to sample volume, (B) the CAT assay to sample volume, and (C) the α-tocopherol standard curve. In the SOD assay, V_1 = basic reaction rate without potato tuber extract, and V_2 = reaction rate with extract. Units of SOD activity are expressed as a function of V_1 and V_2. To generate the standard curve for the α-tocopherol assay, the injection volume was 200 μL and contained 100 μg of α-tocopherol acetate as an internal standard.

potato tubers remained turgid and without sprouts throughout the entire 40 weeks of storage. For clarity, composite representations of the antioxidant and protein data are shown (Fig. 2, A–D). All individual cultivars demonstrated patterns of change similar to the composite representations.

SOD activity was lowest in tubers at zero time storage (Fig. 2A). These tubers had been held in the dark for 2 weeks after harvest at ambient room temperature. During the first 16 weeks of storage at 3°C potato tubers increased their SOD activity ($P < 0.001$) approximately eightfold. After this increase, SOD activity then declined throughout the remaining 24 weeks of storage ($P < 0.001$). Tubers placed into 9°C storage increased their SOD activity during the storage period ($P < 0.001$). Over the course of the entire storage, tubers of all four cultivars stored at 3°C had higher SOD activity than tubers stored at 9°C (Table I). Cultivars differed in SOD activity with Monona $>_{ns}$ Norchip > Russet Norgold $>_{ns}$ Red Pontiac (Table I).

CAT activity was lowest at zero time storage (Fig. 2B). CAT activity then increased at both temperatures more than threefold during the storage period ($P < 0.001$ and $P < 0.001$) (Fig. 2B). Three of the four cultivars had lower CAT activity at 3°C than 9°C (Table I). Cultivars differed in CAT activity with Norchip > Monona > Red Pontiac > Russet Norgold (Table I).

Total soluble protein content of the potato tubers declined during storage at 3 and 9°C approximately 10% relative to preharvest values ($P < 0.001$ and $P < 0.01$) (Fig. 2C). Thus time and temperature dependent changes in SOD and CAT specific activities (data not shown) do not differ appreciably from those shown on a fresh weight basis (Fig. 2, A–B). The protein content of Monona and Norchip tubers did not differ between temperature treatments, while the protein content of Russet Norgold and Red Pontiac tubers was lower during 3°C storage than at 9°C storage (Table I). Cultivars differed in protein content with Norchip $>_{ns}$ Red Pontiac > Russet Norgold > Monona (Table I). Thus, the rank of cultivars for SOD specific activity became Monona > Norchip $>_{ns}$ Russet Norgold $>_{ns}$ Red Pontiac (data not shown). The rank of cultivars for CAT specific activity became Monona > Norchip > Red Pontiac > Russet Norgold (data not shown).

The tubers' content of α-tocopherol was also lowest at zero time storage (Fig. 2D). At both storage temperatures the quantities of α-tocopherol increased approximately fourfold during the storage period ($P < 0.001$ and $P < 0.001$) (Fig. 2D). There was no significant difference between the two temperature treatments (Table I). Cultivars differed in α-tocopherol content with Red Pontiac > Russet Norgold > Monona > Norchip (Table I).

DISCUSSION

Oxygen solubility in distilled water is a function of temperature. There is 40% more dissolved oxygen in distilled water at 9°C than at 25°C, and 16% more at 3°C than at 9°C (10). Potato tubers stored at 3°C undoubtedly contain more dissolved oxygen than tubers stored at 9°C. This is important because electron transport chains leak electrons to oxygen forming superoxide radicals (2, 7, 11, 13–15). The rate of this

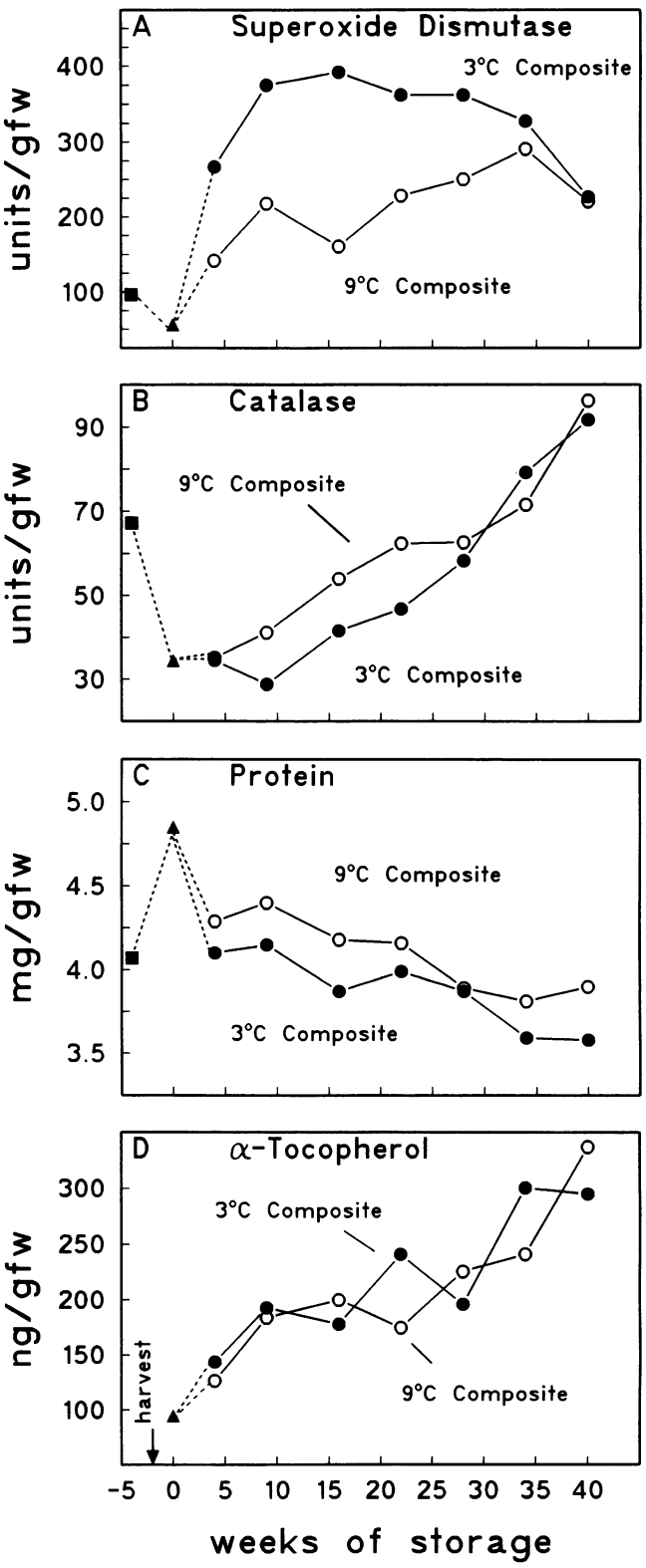

Figure 2. Changes during storage in (A) superoxide dismutase activity, (B) catalase activity, (C) soluble protein content, (D) α-tocopherol content. In (A–D) each symbol is a composite mean of 12 measurements (calculated from three replications of each of the four cultivars). Composite means are from either the preharvest (■), prestorage (▲), 3°C storage (●), or 9°C storage (○) treatments. Note that in (A–D) neither the *x*-axes or *y*-axes begin at 0.

Table I. *Superoxide Dismutase Activity, Catalase Activity, Soluble Protein Content, and α-Tocopherol Content of Potato Tubers Stored at 9 °C and 3 °C*

Values listed are composite means for the entire 40 week storage period. Each composite mean represents 21 measurements (three replications of each cultivar at each of the seven sampling dates during storage). Values with the same letter do not differ significantly ($P < 0.05$). The second column of letters indicates statistical significance when the data from the two temperature treatments were combined.

Cultivar	Storage Temperature	SOD		CAT		Protein		α-Tocopherol	
	°C	units/gfw				mg/gfw		ng/gfw	
Red Pontiac	9	196 c	>b	52.6 de	>c	4.44 a	>a	267 ab	>a
Red Pontiac	3	288 b		54.2 d		4.10 b		287 a	
Russet Norgold	9	194 c	>b	50.0 e	>d	4.08 b	>b	226 c	>b
Russet Norgold	3	299 b		36.3 f		3.73 c		242 bc	
Norchip	9	237 c	>a	72.8 a	>a	4.35 a	>a	146 d	>d
Norchip	3	357 a		66.7 b		4.33 a		141 d	
Monona	9	237 c	>a	68.6 b	>b	3.48 d	>c	212 c	>c
Monona	3	377 a		60.5 c		3.37 d		214 c	

leakage is proportional to the local oxygen concentration (14). Treatments with redox-cycling compounds, ionizing radiation, hyperbaric oxygen, or increasing respiration rates are known to readily increase superoxide anion production and/or levels of antioxidative enzymes in *Escherichia coli* (11, 13, 26), and in some plants (4, 8, 13–15). Of these treatments, exposure to an elevated oxygen concentration may partly explain the increase in SOD activity when potatoes are placed into storage at either 3 or 9°C (Fig. 2A), and the differential induction of SOD activity observed between the two temperatures (Table I).

Alternatively, the increases in SOD and CAT activity may have had no relation to substrate levels. The increases may reflect only an increase in constitutive levels of expression or perhaps a generalized increase in all tuber enzyme activities. However, the latter does not appear to be the case. Several tuber enzymes unrelated to activated oxygen metabolism have been studied during extended term storage (23, 28). Some of the enzyme activities exhibited no appreciable change during storage (UDP-glucose pyrophosphorylase, ribonuclease); others demonstrated time dependent increases in activity (apyrase, sucrose-phosphate synthase); and still others, time dependent decreases in activity (α-amylase, phenol oxidases). Thus, a general elevation of potato tuber enzyme activities would not be expected to accompany the increased SOD and CAT activities during storage.

Surprisingly, in some cases an increase in SOD activity alone has been shown to enhance cytotoxicity by active oxygen species (8, 17, 26). In these instances, cytotoxicity was likely due to accelerated formation of the enzyme's product, hydrogen peroxide, and subsequent generation of the hydroxyl radical by the metal catalyzed Haber-Weiss reaction. It has been suggested that in order to minimize cytotoxicity by activated oxygen in the presence of elevated SOD activity, that hydrogen peroxide must also be effectively scavenged (8, 26). Therefore, concurrent increases in catalases and peroxidases may become necessary.

An analogous situation was observed in potato tubers stored at 3°C. Compared to the zero time measurement there was a dramatic eightfold increase in SOD activity in tubers placed into 3°C storage with no corresponding increase in CAT activity during the early storage period at 3°C (Fig. 2, A–B). While at the intermediate storage temperature of 9°C, the gradual increase in CAT activity more closely paralleled the increase in SOD activity over time (Fig. 2, A–B). Enhanced H_2O_2 scavenging during low temperature storage does not arise from nonspecific peroxidase activity either, because nonspecific peroxidase activity has been observed to decrease in potato tubers stored at 2.5 and 9.5°C (23). The decrease occurred during the early storage season, but after 6 months of storage, nonspecific peroxidase activity did again increase (23). Thus, during storage at 3°C an increased hydrogen peroxide scavenging activity, from either CAT or other peroxidases would not accompany increased SOD activity during early season storage.

It has been shown that high constitutive levels or high induced levels of antioxidants can impart resistance to activated oxygen (6, 12, 13, 15, 30). The cultivars Monona and Norchip may possess such resistance because they possess the highest levels of both SOD and CAT (Table I). Monona and Norchip also maintain the lowest rates of electrolyte leakage, the lowest sugar content, and the best chip color of the four cultivars studied. These features make the two cultivars superior for processing purposes (29). The cultivars with the lowest enzymatic antioxidant capacity were Red Pontiac and Russet Norgold (Table I). Red Pontiac also had the leakiest membranes of all four cultivars, particularly during the 3°C storage treatment when leakage rates increased 252% compared to the 9°C storage treatment (29). The low enzymatic antioxidant content of these cultivars may have rendered them more susceptible to activated oxygen damage.

Damage such as free radical mediated membrane lipid peroxidation or deesterification is very detrimental to membrane function (6, 8, 13–15). Lipid peroxidation in stored potatoes when measured as thiobarbituic acid reactivity has been shown to increase throughout the storage period (20). In

general, increases in lipid peroxidation have been interpreted as both a result and an indicator of an increased activated oxygen content (4, 6, 7, 14, 15, 30). α-Tocopherol protects unsaturated membrane lipids from peroxidation (7, 13, 14, 18, 19, 24, 30) and has been shown to increase in stressed plants (24). In this experiment Red Pontiac and Russet Norgold had the highest α-tocopherol levels (Table I). Perhaps Red Pontiac and Russet Norgold have higher α-tocopherol contents in order to compensate for the low levels of enzymatic antioxidants found in those cultivars (Table I). α-Tocopherol content also increased with the length of time that potato tubers were held in storage (Fig. 2D). The increase occurred while quantities of other tuber membrane lipids were in decline (29), and roughly parallels the increase in potato tuber lipid peroxidation reported by Łojkowska and Hołubowska (20), which would imply an induction of a α-tocopherol to counter the increased lipid peroxidation.

In conclusion, we have documented time, and temperature dependent differences in three antioxidants of stored potato tubers. By analogy to other aerobic systems it is possible that the low temperature and the time dependent increases in SOD, CAT, and α-tocopherol may indicate an increased rate of activated oxygen production in the stored potato tubers. We have also documented cultivar dependent differences in antioxidant content. Monona and Norchip, which have superior processing characteristics out of storage (29), also had the highest levels of SOD and CAT. Perhaps genetic alterations which raise the levels of tuber SOD or CAT, or CAT and SOD concurrently, may improve the ability of potato tubers to withstand oxidative stresses during low temperature and long-term storage.

LITERATURE CITED

1. **Armstrong DA, Buchanan JD** (1978) Reactions of O_2^-, H_2O_2 and other oxidants with sulfhydryl enzymes. Photochem Photobiol **28:** 743–755
2. **Boveris A, Sanchez RA, Beconi MT** (1978) Antimycin- and cyanide-resistant respiration and superoxide anion production in fresh and aged potato tuber mitochondria. FEBS Lett **9:** 333–338
3. **Davies KJA** (1987) Protein damage and degradation by oxygen radicals. I. General aspects. J Biol Chem **262:** 9895–9901
4. **Decleire M, De Cat W, De Temmerman L, Baeten H** (1984) Modifications de l'activité des peroxydase, catalase et superoxyde dismutase dans des feuilles d'épinard traité à l'ozone. J Plant Physiol **116:** 147–152
5. **del Río LA, Ortega MG, López AL, Gorgé JL** (1977) A more sensitive modification of the catalase assay with the Clark oxygen electrode. Application to the kinetic study of the pea leaf enzyme. Anal Biochem **80:** 409–415
6. **Dhindsa RS, Matowe W** (1981) Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. J Exp Bot **32:** 79–91
7. **Elstner EF** (1987) Metabolism of activated oxygen species. *In* DD Davies, ed, The Biochemistry of Plants, Vol 11, Biochemistry of Metabolism. Academic Press, San Diego, pp 253–315
8. **Finazzi-Agro A, Di Giulio A, Amicosante G, Crifo C** (1986) Photohemolysis of erythrocytes enriched with superoxide dismutase, catalase, and glutathione peroxidase. Photochem Photobiol **43:** 409–412
9. **Forman HJ, Fridovich I** (1973) Superoxide dismutase: a comparison of rate constants. Arch Biochem Biophys **158:** 396–400
10. **Forstner H, Gnaiger E** (1983) Appendix A calculation of equilibrium oxygen concentration. *In* E Gnaiger, H Forstner, eds, Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer-Verlag, Berlin, pp 321–333
11. **Fridovich I** (1986) Biological effects of the superoxide radical. Arch Biochem Biophys **247:** 1–11
12. **Gruber MY, Glick BR, Thompson JE** (1990) Cloned manganese superoxide dismutase reduces oxidative stress in *Escherichia coli* and *Anacystis nidulans*. Proc Natl Acad Sci USA **87:** 2608–2612
13. **Halliwell B, Gutteridge JMC** (1985) Free Radicals in Biology and Medicine. Clarendon Press, Oxford
14. **Halliwell B** (1987) Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. Chem Phys Lipids **44:** 327–340
15. **Harper DB, Harvey BMR** (1978) Mechanism of paraquat tolerance in perennial ryegrass. II. Role of superoxide dismutase, catalase and peroxidase. Plant Cell Environ **1:** 211–215
16. **Imlay JA, Linn S** (1988) DNA damage and oxygen radical toxicity. Science **240:** 1302–1309
17. **Iwahashi H, Ishii T, Sugata R, Kido R** (1988) Superoxide dismutase enhances the formation of hydroxyl radicals in the reaction of 3-hydroxyanthranilic acid with molecular oxygen. Biochem J **251:** 893–899
18. **Larson RA** (1988) The antioxidants of higher plants. Phytochemistry **27:** 969–978
19. **Liebler DC, Kling DS, Reed DJ** (1986) Antioxidant protection of phospholipid bilayers by α-tocopherol. Control of α-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. J Biol Chem **261:** 12114–12119
20. **Łojkowska E, Hołubowska M** (1989) Changes of the lipid catabolism in potato tubers from cultivars differing in susceptibility to autolysis during the storage. Potato Res **32:** 463–470
21. **Monk LS, Davies HV** (1989) Antioxidant status of the potato tuber and Ca^{2+} deficiency as a physiological stress. Physiol Plant **75:** 411–416
22. **Niehaus WG Jr** (1978) A proposed role of superoxide anion as a biological nucleophile in the deesterification of phospholipids. Bioorg Chem **7:** 77–84
23. **Nowak J** (1977) Biochemical changes in stored potato tubers with different rest periods. II. Influence of the storage temperature and isopropyl phenylcarbamates on enzyme activities. Z Pflanzenphysiol **81:** 125–140
24. **Price A, Hendry G** (1987) The significance of the tocopherols in stress survival in plants. *In* C Rice-Evans, ed, Free Radicals, Oxidant Stress and Drug Action. Richelieu Press, London, pp 443–450
25. **Rastovski A** (1987) Storage losses. *In* A Rastovski, A van Es, eds, Storage of Potatoes: Post Harvest Behaviour, Store Design, Storage Practice, Handling. PUDOC, Wageningen, pp 177–180
26. **Scott MD, Meshnick SR, Eaton JW** (1987) Superoxide dismutase-rich bacteria. Paradoxical increase in oxidant toxicity. J Biol Chem **262:** 3640–3645
27. **Snyder JC, Desborough SL** (1978) Rapid estimation of potato tuber total protein content with Coomassie brilliant blue G-250. Theor Appl Genet **52:** 135–139
28. **Sowokinos JR** (1990) Stress-induced alterations in carbohydrate metabolism. *In* M Vayda, W Park, eds, Molecular and Cellular Biology of the Potato. C.A.B. International, Wallingford, United Kingdom pp 137–158
29. **Spychalla JP, Desborough SL** (1990) Fatty acids, membrane permeability and sugars of stored potato tubers. Plant Physiol **94:**1207–1213
30. **Wise RR, Naylor AW** (1987) Chilling-enhanced photooxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. Plant Physiol **83:** 278–282